The Biological Response of Infant Nonhuman Primates to a Polychlorinated Biphenyl

by L. J. Abrahamson* and J. R. Allen*

Recently there have been numerous reports on lesions produced by the polychlorinated biphenyls (PCBs) in various animals. However, there is a paucity of data on the effects of these compounds in primates, including man. The PCBs have been found in measurable amounts in human adipose tissue (*1*) and in human (*2*) and cows' milk (*3*), where they pose a source of contamination for infants. In 1968, over 1000 persons were affected following the consumption of PCB-contaminated rice oil (*4*). The ingestion of approximately 2.0 g of PCB caused nausea, lethargy, subcutaneous edema of the face, and acneform lesions in a large percentage of the exposed persons. Adolescents and young adults were affected most severely, and the symptoms and lesions have persisted in many of these persons for over 3 years. Infants born of affected mothers were small and had brown stained skin and eye discharge. Male school children who were exposed to the PCBs were below average in height and weight.

Allen et al. (*5,6*) have recently fed PCBs to adult rhesus monkeys at a level of 300 ppm in the diet. These animals developed alopecia, facial edema, and isolated acneform lesions of the face within 1 month. In addition to bone marrow hypoplasia, liver hypertrophy, and increased hepatic microsomal enzyme activity, they had extensive gastric hyperplasia, dysplasia, and invasion of the underlying submucosa of the stomach.

There are data which indicate that fetal and neonatal animals are much more susceptible to the effects of the PCBs than are adult animals. Low mating indices and decreased survival of pups had been reported in rats fed PCBs (*7*). Birds fed the PCBs produced thin-shelled eggs which hatched poorly and gave rise to offspring that suffered teratogenic abnormalities (*7-10*). The PCBs have been blamed for premature births and early deaths of California sea lion pups (*11*), and breeding mink and their offspring were very sensitive to these compounds (*12*). In contrast to the above, the presently reported study shows that the infant rhesus monkey is able to survive without exhibiting any overt symptoms of PCB intoxication when given doses sufficiently large to cause severe morbidity in adult animals.

Materials and Methods

Nine rhesus monkeys, seven males and two females, were separated from their mothers at 1 month of age. The infants were placed in a nursery in individual wire mesh cages equip-

*Department of Pathology, University of Wisconsin Medical School, Madison, Wisconsin 53706 and Regional Primate Research Center, University of Wisconsin, Madison, Wisconsin 53706.

ped with surrogate mother, diapers, and feeding clip positioned to hold a 180-cc bottle at a height and angle conducive to feeding. The animals were offered fresh Similac formula (Ross Laboratories, Columbus, Ohio; prepared at the recommended dilution of 132 g Similac powder per liter of reconstituted formula) twice daily, two pieces of Purina Monkey Chow (Ralston-Purina Company, St. Louis, Missouri) and half of an orange daily. Records were compiled on food intake and weight gain throughout the course of the experiment. Five of the infant monkeys, four males and one female, were intubated daily with 35 mg/kg of the PCB Aroclor 1248 (Monsanto Company, St. Louis, Missouri). Also administered as a diluent for the Aroclor were 2 ml/kg of corn oil. Two additional infants, both males, received 2 ml/kg of corn oil while the remaining two infants, one male and one female, received no intubated material. Blood was drawn at the beginning of the experiment, at two weeks and at the end of the fourth week just prior to sacrifice. Hematocrit, hemoglobin, total white cell count, differential white count, serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, blood urea nitrogen, total serum protein, and serum electrophoresis were established on all blood samples. After 30 days the animals were starved overnight, anesthetized, and exsanguinated by severing the jugular veins. Portions of the tissues were placed in 10% neutral buffered formalin for 24 hr, dehydrated, embedded in paraffin, sectioned at 5 μ and stained with hematoxylin and eosin for histologic evaluation. For electron microscopic evaluation, portions of the liver were cut into small cubes and fixed for 2 hr at 0°C in 1.33% osmium tetroxide buffered in 0.67*M* S-collidine at pH 7.41 (*13*). Tissues were then dehydrated through a graded series of ethanol and embedded in an epoxy resin mixture (*14*). Sections of the tissues were cut on an ultramicrotome, placed on uncoated copper grids, stained with uranyl acetate, and examined with an electron microscope.

The remaining portion of the liver was chopped into small pieces and homogenized in two volumes of 0.25*M* sucrose with 0.010*M* $MgCl_2$ and 0.015*M* KCl at 0°C by using a Potter homogenizing tube and a Teflon pestle. Levels of protein (*15*) and RNA and DNA (*16,17*) were determined on the homogenates. A postmitochondrial supernatant was obtained by centrifugation at 15,000*g* for 10 min. The pellet was recentrifuged under the same conditions after one wash with sucrose solution. The two supernatants were combined and spun at 105,000g_{max} for 110 min to pellet the microsomes. The pellets were washed in sucrose solution and repelleted for 80 min. Resulting pellets were resuspended in sucrose solution equal to three volumes of liver and stored at -70°C. Enzyme levels of aniline hydroxylase (*18,19*), nitroreductase (*18,20,21*), *N*-demethylase (*18,22,23*), glucose-6-phosphatase (*24,25*) and *p*-nitrophenyl acetate hydrolase (esterase) (*26*) were determined, as were levels of microsomal protein (*15*), RNA (*16*), phospholipids (*25,27*), and cholesterol (*27-29*).

Data were expressed as mean values ± one standard deviation and were analyzed by using Student's *t* test.

Results

Throughout the period of examination all animals appeared healthy and exhibited normal behavioral patterns. However, the food consumption of the PCB-fed animals was slightly less than that of the control animals. In addition, the experimental animals did not gain weight as rapidly as did the controls (weight gain of 120 g/animal for experimentals and 256 g/animal for controls).

The clinical data obtained during the course of the experiment was unaltered in the PCB-fed animals with the exception of the hemoglobins and hematocrits. In three of the five PCB-fed animals there was a decrease in hematocrit of 3.1% and a corresponding decrease in the hemoglobin level, while the hematocrit level of control animals increased 4.8%, approaching normal adult values with corresponding hemoglobin increases.

At necropsy there was found to be a decided hypoplasia of the thymus of the PCB-fed animals (Table 1). The experimental animals also exhibited a slight increase in weight of the spleen, adrenals, liver and brain when expressed in relation to body weight. When the tissues were examined by light microscopy, the thymus showed a regression of the cortical areas, and the bone marrow was hypoplastic with a decrease particularly in the erythroid series. In addition, one of the PCB-fed animals had keratin cysts on the upper eyelids and hypertrophy of the epithelium of the hair follicles. This animal also showed a moderate edema of the gastric mucosa, while another PCB-fed animal had focal hyperplasia of the gastric mucosa.

When the liver tissue was examined by electron microscopy, the reasons for the increase in liver size were apparent. There was a moderate proliferation of the smooth edoplasmic reticulum and random collections of lipid droplets within the cytoplasm of the affected cells. The other cytoplasmic organelles appeared to be unaffected.

Table 1. Organ weights of infant rhesus monkeys fed Aroclor 1248.

	Organ weights, % of body weight	
	Control[a]	Experimental[a]
No. of animals	4	5
Liver	2.62 ± 0.28	3.19 ± 0.39[b]
Thymus	0.298 ± 0.033	0.093 ± 0.059[c]
Adrenal	0.050 ± 0.009	0.080 ± 0.017[b]
Spleen	0.118 ± 0.025	0.151 ± 0.045
Brain	9.01 ± 0.45	10.46 ± 1.05[b]
Pancreas	0.07 ± 0.01	0.09 ± 0.02
Lungs	0.86 ± 0.12	1.01 ± 0.14
Heart	0.53 ± 0.02	0.48 ± 0.09
Kidney	0.62 ± 0.09	0.63 ± 0.02
Thyroid	0.032 ± 0.015	0.034 ± 0.008

[a]Values expressed as means ± 1 standard deviation.
[b]Difference with controls statistically significant: $p < 0.05$.
[c]Difference with controls statistically significant: $p < 0.001$.

There were also changes in the biochemical composition of the liver tissue (Table 2). As a result of the hypertrophy of the cytoplasmic components of the cell there was a decrease in DNA per gram liver. There was also an increase in the concentration of RNA/DNA, while the protein/DNA levels were not affected appreciably in the liver homogenates of the PCB-fed infants.

Table 2. Compositional data on liver homogenates prepared from infant rhesus monkeys fed Aroclor 1248.

	Control[a]	Experimental[a]
No. of animals	4	5
DNA, mg/g liver	4.45 ± 0.36	3.72 ± 0.22[b]
RNA, mg/mg DNA	1.53 ± 0.20	1.85 ± 0.08[b]
Protein, mg/mg DNA	96.9 ± 7.8	95.3 ± 10.3

[a]Values expressed as means ± 1 standard deviation.
[b]Difference with controls statistically significant: $p < 0.02$.

When the biochemical data obtained on the hepatic microsomes were expressed in relation to microsomal protein (to show activity and composition per unit of membrane) there was an increase in RNA, phospholipid and cholesterol. However, statistically these increases were not sufficiently great to be significant. Levels of enzymatic activity were also not affected appreciably (Table 3).

Discussion

There was considerable variation in the response of infant and adult monkeys to the PCBs. Within a month following the ingestion of a diet containing 300 ppm of a PCB, adult monkeys developed alopecia, acne, subcutaneous edema, bone marrow hypoplasia, anemia, relative leukocytosis, and hyperplasia of the gastric epithelium. The decided liver hypertrophy was associated with a proliferation of the smooth endoplasmic reticulum and an increased enzymatic activity of the microsomal fraction. When infant monkeys were fed similar doses of PCBs for one month they were free of acne, alopecia, subcutaneous edema, and the changes in the bone marrow, stomach, and liver were not of the same magnitude as that present in adult monkeys. There was only a moderate hypertrophy of the endoplasmic reticulum and an absence of any appreciable increase of microsomal enzyme activity in the infant monkey

Table 3. Compositional and enzymatic data on liver microsomes isolated from infant rhesus monkeys fed Aroclor 1248.

	Control[a]	Experimental[a]
No. of animals	4	5
Protein, mg/g liver	26.9 ± 4.8	25.6 ± 2.7
RNA, μg[b]	84.2 ± 14.8	89.9 ± 10.9
Phospholipid, μg[b]	330 ± 80	402 ± 60
Cholesterol, μg[b]	11.4 ± 1.5	12.8 ± 1.5
Aromatic hydroxylase, nmole *p*-aminophenol/30 min[b]	27.4 ± 10.9	23.1 ± 11.6
N-Demethylase, nmole formaldehyde/30 min[b]	87.7 ± 7.2	91.9 ± 31.4
Nitroreductase, nmole *p*-aminobenzoate/hr[b]	18.8 ± 6.9	17.3 ± 4.5
Glucose-6-phosphatase, μmole PO_4/15 min[b]	3.82 ± .43	2.94 ± 1.17
Esterase, μmole *p*-nitrophenyl/min[b]	2.39 ± .48	2.00 ± .77

[a]Values expressed as means ± 1 standard deviation.
[b]Values expressed per mg of microsomal protein.

livers. In addition, the bone marrow showed only a moderate decrease in the erythroid elements, and the stomach of a single animal had isolated areas of gastric mucosal hyperplasia.

The reasons for this variation in response of two age groups of nonhuman primates to the PCBs remain to be clarified. Undoubtedly, the differences in absorption, metabolism, storage, or excretion of the PCBs are instrumental in determining the degree of toxicity experienced by these animals. If infant monkeys were unable to absorb the compounds as efficiently as adults, this would be an explanation for the lower tissue levels (L. J. Abrahamson and J. R. Allen, unpublished observations) and reduced toxicity. However, adult monkeys, like rats (*30*), are able to absorb a large percentage of the ingested PCBs. If, in fact, the infants are unable to absorb as much of the PCBs as the adults, consideration will then have to be given to factors that might be responsible, such as a more rapid movement of a fluid diet through the gastrointestinal tract, reduced absorptive surface of the infant monkey intestine, or lack of enzymes necessary for absorption of hydrophobic compounds.

The precise role played by the metabolites of PCBs in monkeys is not clear. Whether a majority of the compound is absorbed and excreted unmodified or the metabolism of these compounds is an important aspect of their storage and excretion is unclear. However, in rats the PCBs cause a decided proliferation of the smooth endoplasmic reticulum and an increase in microsomal enzyme activity (*31,32*). These modifications in enzyme activity may be related to the metabolism of the PCBs. It has been demonstrated by chromatographic procedures that the PCB residues in the body tissues and excreta of rats are decidedly different from those shown in chromatograms of the initial PCBs (*33,34*). In addition, West et al. (*35*) have isolated a number of metabolites from the urine of biphenyl-fed rats. It is possible that the metabolism of the PCBs also occurs in the livers of adult monkeys since they, too, have a proliferation of the endoplasmic reticulum and an increase in microsomal enzyme activity following exposure to these chlorinated hydrocarbons. The lack of any appreciable biochemical change in the endoplasmic reticulum or modifications in the enzymatic activities of the liver of infant monkeys exposed to the PCBs suggest that the metabolism of these compounds is less than that present in adult monkeys. The inability of the infant monkey liver to metabolize the PCBs may be one explanation for their greater tolerance for the compounds.

In addition to the PCBs, infant monkey livers do not respond as readily, as do adult monkey livers, to known enzyme inducers such as the antioxidants butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) (*36*). Previous experiments have also shown that the hepatic microsomes from infant monkeys have low activity of enzymes responsible for *N*-demethylation and dehydrogenation (*37*). These data suggest that the inability of the infant monkey liver to respond to enzyme inducers and readily to metabolize foreign substances such as the

PCBs results from the lack of maturation of the particular system.

Undoubtedly, tissue levels of the PCBs are directly related to the lesions that develop in the experimental animals. The possibility exists that the tissues of the infant monkeys may not be able to store the absorbed PCBs as readily as the tissues of adult monkeys. Since the major storage depot for the absorbed PCBs is in the adipose tissue and the infant monkey has little fat, there are fewer cells in which to deposit these compounds. A second, seemingly very important storage depot for the PCBs may be in the membranes of the hepatic endoplasmic reticulum. Recently, Norback et al. (38) have shown that the liver microsomes of rats fed highly chlorinated dibenzo-*p*-dioxin have a high concentration of this hydrophobic chlorinated aromatic hydrocarbon 6 weeks after the compounds have been removed from the diet. Such may also be the case in the liver of monkeys exposed to the PCBs. Since the infant monkey liver shows only minimal proliferation of the hepatic endoplasmic reticulum regardless of the inducer, the storage capacity for PCBs within the membranes of the endoplasmic reticulum would not be nearly as great as that of the proliferated endoplasmic reticulum in the livers of the PCB-fed adult monkeys.

The data presented in this report indicate that the infant monkey is able to tolerate doses of the PCBs that produce extreme morbidity in adult monkeys. The precise reason for this variation in response is unclear. There have been a number of possibilities proposed, such as variation in absorption, metabolism, storage, or excretion of the PCBs by adult and infant monkeys. However, it will be the subject of future research to determine which, if any, of the above is responsible for these differences.

Acknowledgements

The assistance of Joan Scheffler, Susan Edgerton, Adrienne Cappas, and Jonathan Engblom is gratefully acknowledged. This investigation was supported in part by U. S. Public Health Service grants ES-00472 and RR-00167 from the National Institutes of Health, and the University of Wisconsin Sea Grant. Primate Center Publication No. 12-016.

REFERENCES

1. Yobs, A. R. Levels of polychlorinated biphenyls in adipose tissue of the general population of the nation. Environ. Health Perspec. 1: 179 (1972).
2. Westöö, G., Noren, K. E., and Andersson, M. Var Föda *10* (1970); reported in Edwards, R. The polychlorinated biphenyls, their occurrence and significance: a review. Chem. Ind. (London): 1340 (1971).
3. Fries, G. F. Polychlorinated biphenyl residues in the milk of environmentally and experimentally contaminated cows. Environ. Health Perspec. 1: 55 (1972).
4. Kuratsune, M. An epidemiologic study on "Yusho" or chlorobiphenyls poisoning. Fukuoka Acta Medica 60: 513 (1969).
5. Allen, J. R., and Norback, D. H. Polychlorinated biphenyl and triphenyl induced gastric mucosal hyperplasia in primates. Science 179: 498 (1973).
6. Allen, J. R. Abrahamson, L. J., and Norback, D. H. Biological effects of polychlorinated biphenyls and triphenyls on the subhuman primate. Environ. Res. in press.
7. Keplinger, M. L. et al. Toxicological studies with polychlorinated biphenyls. Proceedings of the NIEHS Polychlorinated Biphenyl Meeting, December 20-21, 1971.
8. Dahlström, N., as quoted by Tinker, J. The PCB story: seagulls aren't funny anymore. New Scientist Science J. 16 (April 1, 1971).
9. McLaughlin, J., Jr., et al. The injection of chemicals into the yolk sac of fertile eggs prior to incubation as a toxicity test. Toxicol. Appl. Pharmacol. 5: 760 (1963).
10. Hays, H., and Risebrough, R. W. Natural History, November 1971; as reported: Controversy continues over PCB's. Chem. Eng. News 49: 32 (1971).
11. Gilmartin, W. G. DDT and PCB residues in parturient California sea lions in relation to premature or normal birth. Paper presented at seminar, Center for Environmental Toxiocology, University of Wisconsin, March 14, 1972.
12. Ringer, R. K., Aulerich, R. J., and Zabik, M. Effect of dietary polychlorinated biphenyls on growth and reproduction of mink. Paper presented to Division of Water, Air and Waste Chemistry, American Chemical Society Meeting, New York, Aug. 28–Sept. 1, 1972; Proceedings 12: 149 (1972).
13. Bennett, H. S., and Luft, J. H. S-Collidine as a basis for buffering fixatives. J. Biophys. Biochem. Cytol. 6: 113 (1959).
14. Mollenhauer, H. H. Plastic embedding mixtures for use in electron microscopy. Stain Technol. 39: 111 (1964).

15. Lowry, O. H., et al. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265 (1951).
16. Munro, H. N., and Fleck, A. The determination of nucleic acids. In: Methods of Biochemical Analysis. D. Glick, Ed., Interscience, New York-London, Vol. 14, 1966, p. 113.
17. Ceriotti, G. A microchemical determination of desoxyribonucleic acid. J. Biol. Chem. **198**: 297 (1952).
18. Gram, T. E., Rogers, L. A., and Fouts, J. R. Further studies on the metabolism of drugs by subfractions of hepatic microsomes. J. Pharmacol. Exp. Therap. **155**: 479 (1967).
19. Kato, R., and Gillette, J. R. Effect of starvation on NADPH-dependent enzymes in liver microsomes of male and female rats. J. Pharmacol. Exp. Therap. **150**: 279 (1965).
20. Fouts, J. R., and Brodie, B. B. The enzymatic reductions of chloramphenicol, *p*-nitrobenzoic acid and other aromatic nitro compounds in mammals. J. Pharmacol. Exp. Therap. **119**: 197 (1957).
21 Bratton, A. C., and Marshall, E. K., Jr. A new coupling component for sulfanilamide determination. J. Biol. Chem. **128**: 537 (1939).
22. Cochin, J., and Axelrod, J. Biochemical and pharmacological changes in the rat following chronic administration of morphine, nalorphine and normorphine. J. Pharmacol. Exp. Therap. **125**: 105 (1959).
23. Nash, T. The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochem J. **55**: 416 (1953).
24. Harper, A. E. Glucose-6-phosphatase. In: Methods of Enzymatic Analysis. H. U. Bergmeyer, Ed., Academic Press, New York, 1965.
25. Baginski, E. S., Foa P. P., and Zak, B. Microdetermination of inorganic phosphate, phospholipids, and total phosphate in biologic materials. Clin. Chem. **13**: 326 (1966).
26. Norback, D. H., and Allen, J. R. Chlorinated aromatic hydrocarbon induced modifications of the hepatic endoplasmic reticulum: concentric membrane arrays. Environ. Health Perspec. **1**: 137 (1972).
27. Folch, J., Lees, M., and Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. **226**: 497 (1957).
28. Shin, Y. S., and Lee, J. C. Microdetermination of cholesterol and phospholipid in cerebrospinal fluid and serum by silicic acid column chromatography. Clin. Chem. **8**: 598 (1962).
29. Glick, D., Fell, B. F., and Sjolin, K. E. Spectrophotometric determination of nanogram amounts of total cholesterol in microgram quantities of tissue or microliter volumes of serum. Anal. Chem. **36**: 1119 (1964).
30. Albro, P. W., and Fishbein, L. Intestinal absorption of polychlorinated biphenyls in rats. Bull. Environ. Contam. Toxicol. **8**: 26 (1972).
31. Allen, J. R., and Abrahamson, L. J. Morphological and biochemical changes in the liver of rats fed chlorinated biphenyls. Arch. Environ. Contam. Toxicol. in press.
32. Litterst, C. L., et al. Effect of polychlorinated biphenyls on hepatic microsomal enzymes in the rat. Toxicol. appl. Pharmacol. **23**: 112 (1972).
33. Curley, A., et al. Polychlorinated biphenyls: distribution and storage in body fluids and tissues of Sherman rats. Environ. Res. **4**: 481 (1971).
34. Grant, D. L., Phillips, W. E. J., and Villeneuve, D. C. Metabolism of a polychlorinated biphenyl (Aroclor 1254) mixture in the rat. Bull. Environ. Contam. Toxicol. **6**: 102 (1971).
35. West, H. D., et al. The fate of diphenyl in the rat. Arch. Biochem. Biophys. **60**: 14 (1956)
36. Allen J. R., and Engblom, J. F. Ultrastructural and biochemical changes in the liver of monkeys given butylated hydroxytoluene and butylated hydroxyanisole. Food Cosmet. Toxicol. **10**: 769 (1972).
37. Allen, J. R., and Chesney, C. F. Effect of age on the development of cor pulmonale in nonhuman primates following pyrrolizidine alkaloid intoxication. Exp. Molec. Path. **17**: 220 (1972).
38. Norback, D. H., and Engblom, J. F. Chlorinated dibenzo-*p*-dioxin distribution within rat tissues and subfractions of the liver. Fed. Proc. **32**: 236 (1973).